Repellent, irritant and toxic effects of essential oil constituents on *Bemisia tabaci* (Gennadius)

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Master Biological Sciences, track Ecology and Evolution

October, 2012 – March, 2013

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LA RECHERCHE AGRONOMIQUE POUR LE DÉVELOPPEMENT

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Abstract

Whiteflies, *Bemisia tabaci*, are a widespread pest in agriculture, causing crop loss up to 100% by direct and indirect damage. Controlling this pest has proven difficult due to the fact that they stay underside the leaves and their ability to become resistant to conventional pesticides rapidly. Also, environmental and health concerns associated with the use of synthetic pesticides are rising. Therefore, new methods should be investigated to protect crops from *B. tabaci*. Insect proof nets (IPNs) create a physical barrier between the crop and a pest insect, but this alone is not suitable against small insects like *B. tabaci*. A possible solution is combining insect proof nets (IPN) with a naturally occurring repellent. Essential oils, mixtures of volatile secondary metabolites of plants, have been shown to have repellent and toxic abilities against many pest insects, including whiteflies. This study investigates the role of the different compounds in four essential oils; Cumin (*Cuminum cyminum*), Cinnamon (*Cinnalonum zeylanicum*), Lemongrass (*Cymbopogon citratus*) and Citronella grass (*Cymbopogon winterianus*), to see if the constituents are toxic, repellent or irritant. This is also used as a screening for compounds that seem promising to be used in combination with IPNs in the field. This should be a highly repellent, but not very toxic compound, to repel *B. tabaci* but with a reduced risk of the fast development of resistance. In this study I found that most constituents of essential oils have one dominant role (i.e. they are for example toxic or repellent but not both) and the effects of these different compounds combined add up in the mixture of the essential oil. As for the essential oil of citronella grass, this oil is less toxic than some of its individual compounds, suggesting interactions between the compounds when mixed. The most promising compounds to be used against *B. tabaci* in the field, based on their high repellency and low toxicity, are cinnamaldehyde (repellent at <0.084 mg/L and toxic at 8.4 mg/L) and linalool (repellent at 0.006 mg/L but with unknown toxicity).

Introduction

The whitefly, *Bemisia tabaci*, is a serious pest in agriculture. It has a wide host range and is widespread in tropical and subtropical areas all over the world. The damage caused by this pest can be direct; the sucking of the plant sap by the whitefly causes the plant to be weaker and earlier wilting. Equally important is the indirect damage; a black sooty-mold (*Cladosporium* spp. and *Alternaria* spp.) can develop on the honeydew excreted by the whiteflies, causing tackiness and dirt of the plant surface and this might greatly reduce photosynthesis and their commercial value. Moreover, *B. tabaci* is a vector of 111 plant viruses, causing damage and crop yield losses up to 100%. A relatively small population of whitefly can be already sufficient to cause severe damage (Berlinger 1986; Cohen and Berlinger 1986; Blackmer and Byrne 1993; Jones 2003).

Protecting crops from whiteflies with pesticides is difficult. There are some challenges involved in preventing and controlling infestation of whiteflies on host plants. One of these is caused by the fact that whiteflies stay on the underside of leaves, making them less accessible for insecticide foliar sprays. But more importantly, they have developed a high resistance to many conventional pesticides used in agriculture (Roditakis et al. 2009; Elbert and Nauen 2000; Palumbo, Horowitz, and Prabhaker 2001; Denholm et al. 1998). There are several factors that contribute to this high resistance in whiteflies. One is the way in which the insecticides are being used (e.g. excessive and all-year-round use and usage in greenhouses). But *B. tabaci* also has some biological characteristics that give them a high chance of becoming resistant to pesticides; they have a high reproductive rate and a haplodiploid breeding system (Byrne and Devonshire 1996) which is associated with a high potential to become resistant to pesticides (Denholm et al. 1998). New insecticides can be developed to which *B. tabaci* is not resistant yet, but there are other approaches that can possible protecting crops that do not have the problem of resistance as much as conventional pesticides.

Using insecticides comes with some difficulties. Growing concerns about the risks involved with using traditional synthetic insecticides are causing an increased interest in and popularity of more environmentally friendly alternatives, the so called biopesticides. Biopesticides are considered to be less harmful to the environment and health because they consist of natural products. Among them are essential oils, mixtures of volatile compounds that are produced as secondary metabolites, which are derived from aromatic plants. They have been of interest as a more environmentally friendly alternative for the last decade. Most research on them has so far been done on pests of stored products (Regnault-Roger 1997; Regnault-Roger, Vincent, and Arnason 2012; Isman and Machial 2006).

A way to protect plants from pest insects without the usage of pesticides, is by creating a physical barrier between the insect and the plant. This can be accomplished with insect proof nets (IPN), or insect proof screens. IPNs have been used successfully to prevent crops from damage caused by phytophagous insects under laboratory conditions and in greenhouses (Berlinger et al. 2002). In Benin, IPNs have been used against pest insects in the field; nets with large mesh sizes have been shown to protect against the diamondback moth (*Plutella xylostella*) and other Lepidoptera, even when compared with a treatment of foliar insecticide spray the IPNs where more effective (Martin et al. 2006).

However against smaller insects, such as whiteflies and mites, nets with relative large meshes do not provide protection, as the insects can cross them. The application of those nets can even make the situation worse, as they create a protected area for the small insects in which (larger) predators are not able to reach them. Although the application of nets with smaller mesh sizes has proven effective in reducing the infestation of whiteflies when applied at the entrance of greenhouses (Teitel 2007; Taylor et al. 2001), application in the field is hindered by their effect on the microclimate. The lack of airflow through the nets and the higher temperature and/or ambient humidity makes the usage of those nets unfavorable. To protect crops against the different types of pests, including the smaller insects, another approach is needed. The use of IPNs with larger mesh size in combination with a substance (i.e. an insecticide or repellent) that acts against small insects (e.g. whiteflies) might be a solution. This way the IPNs will provide a physical border against large pest insects, while the substance prevents small insects from infesting the plants.

Such a substance can prevent insects from coming to the plants and passing through the net in three different ways; it can be toxic, repellent, or both. Furthermore, a substance can be repellent in two different ways; it can be spatially repellent (i.e. repellent from a distance) or repellent after contact (i.e. the substance is irritant). For the application of a plant derivative in combination with IPNs, a spatial repellent product would be most favorable against *B. tabaci*. This would prevent them from coming to the plants, without the need of the insect to actually be near and having to touch the net (which would increase the risk of infestation). Also, since the substance is not toxic, it might reduce the risk of the whiteflies to quickly evolve resistance to the compound.

Many studies on many pest insects have shown repellent and toxic effects in different essential oils (Isman 2000; Regnault-Roger et al. 1993; Shaaya et al. 1991; Zhang, McAuslane and Schuster 2004; Nerio et al. 2010). The mode of action of some essential oils have been studied in the cockroach *Periplaneta americana* and the fruit fly *Drosophila melanogaster* (Enan 2005). Despite this, very little is known about the way essential oils influence insects at the molecular level. It cannot be automatically assumed that naturally occurring products are always safe to use. When using natural products, there are still risks involved concerning health and environment, especially when used in high quantities. These risks are better estimated and tested on individual compounds than on mixtures, such as essential oils. Therefore, an individual compound has the preference over a mixture to be used as a repellent on a IPN.

Prior to this study, essential oils from 20 plants were tested for their toxic, spatial repellent and irritant effects on *B. tabaci* (Martin and Deletre, unpublished). From those, four where selected for this study, based on their strong toxic and repellent properties. These are the essential oils of Cumin (*Cuminum cyminum*), Cinnamon (*Cinnalonum zeylanicum*), Lemongrass (*Cymbopogon citratus*) and Citronella grass (*Cymbopogon winterianus*). To determine the individual constituents of essential oil, they were analyzed using gas chromatograph-mass spectrometry. This data was used to select the most abundant constituents in each of the four essential oil, and these compounds were tested for their toxicity, spatial repellency and irritancy using different bioassays in the laboratory. Two positive controls were also tested in the same way: N,N-diethyl-m-toluamide (DEET) and permethrin. The positive controls were chosen based on their known toxic and repellent effects on many insect species (Brown and Hebert 1997).

The structural formulas of the tested compounds, as well as of DEET and permethrin, are shown in figure 1 . All essential oil constituents tested in this study belong to a group called terpenes. The compounds, including DEET and permethrin, are organic molecules with similar structures; most of them contain a benzene ring or a less saturated ring structure, combined with (in most cases) ester, ether or aldehyde groups.

This study is a screening for compounds with a high potential to be used in combination with IPNs to protect crops from *B. tabaci*. The fact that *B. tabaci* can become resistant to toxic compounds rapidly, a substance with a high spatial repellency, but a low toxicity would be preferred. Also, since the effect of the mixture (i.e. the essential oil) is known, when the effects of the individual compounds have been determined, this gives information about the way the substances act together in the mixture.

Table 1. List of most abundant compounds in the essential oils of cumin, cinnamon, lemongrass and citronella grass. Their relative volume percentages in which they were found using GC-MS analysis are also given.

Cumin	**Cinnamon**	**Lemongrass**	**Citronella grass**
Cuminaldehyde (30.09%)	Cinnamaldehyde (78.51%)	Citral (74.08%)	Citronellal (34.74%)
β-pinene (12.19%)	2-metoxycinnamaldehyde (9.65%)	Geraniol (4.5%)	Geraniol (22.50%)
γ-terpinene (11.59%)	Cinnamylacetate (3.15%)	Limonene (1.9%)	Citronellol (12.03%)
P-cymene (9.74%)		β-caryophyllene (1.8%)	Geranyl acetate (3.51%)
		Linalool (0.69%)	Limonene (3.34%)

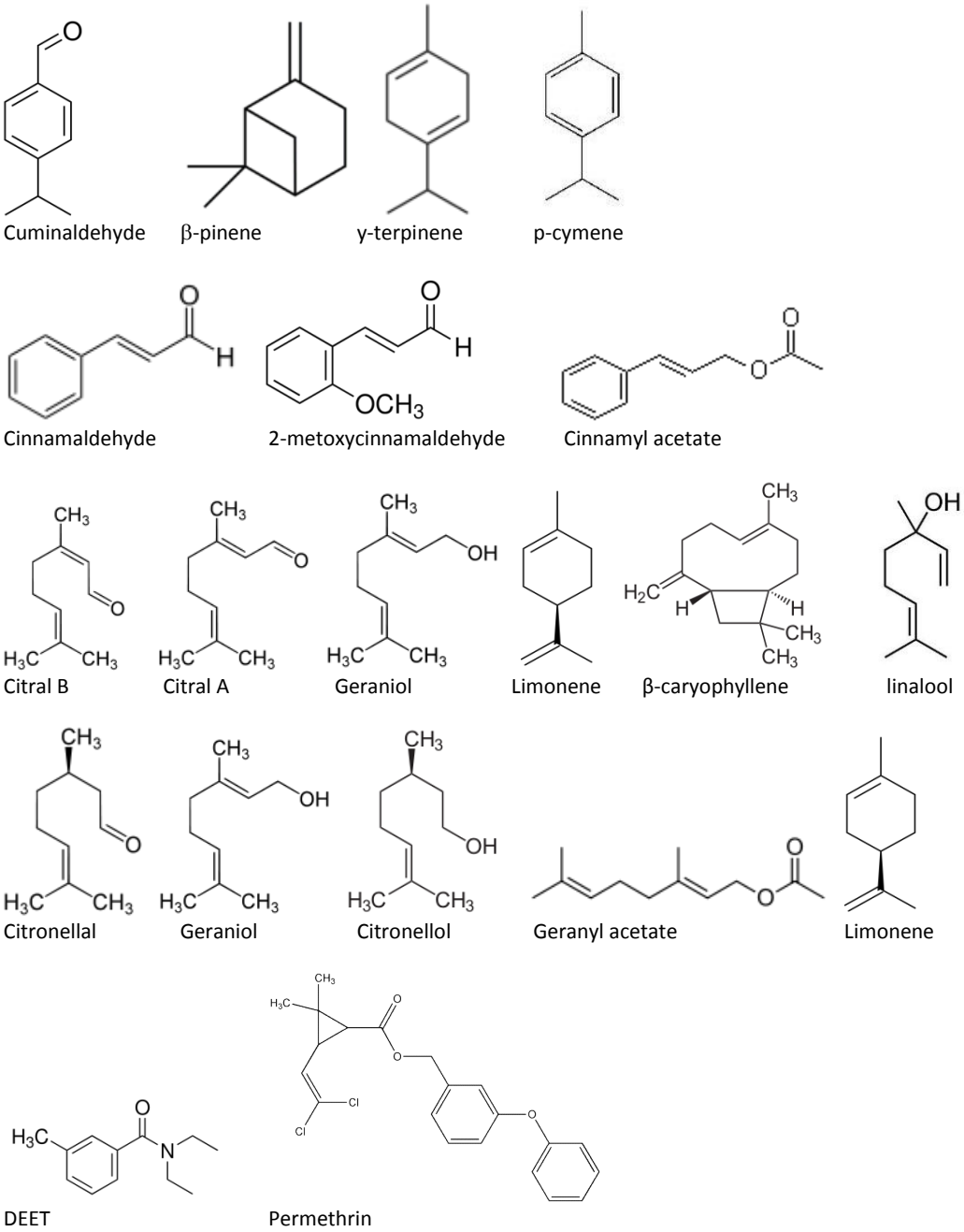


Figure 1. Molecular structures of the tested compounds.

Material and Methods

Insects

Bemisia tabaci biotype Q (MPL strain) was reared on tomato plants (*Solanum lycopersicum* L.) in two cages, one plant per cage. The cages were kept in a room at a constant temperature of 27°C ± 1°C, 50% ± 10% humidity and a light/dark period of 12:12h. The plants in the cage were replaced every Monday, Wednesday and Friday. The old plants were preserved in the same room as the cages, so the eggs on them could develop. On the same day as the plants were changed, leaves from tomato plants with eggs of approximately 3 weeks old were put in the cages to hatch. This way a good population of whiteflies was maintained.

Compounds

A total of 15 different compounds originating from 4 essential oils were tested. DEET and Permethrin were tested as positive controls. All compounds were commercially obtained. The concentrations at which the compounds were tested were based on the concentrations at which they were found in the essential oil of origin. This was determined using gas chromatography-mass spectrometry (GC-MS) prior to the experiments. Unless otherwise specified, compounds were diluted in 97% ethanol to the same concentration as they were found in a 1% essential oil (the '1% solution`). Also a 10-times dilution (the '0.1% solution') of this was tested for most bioassays. If a compound was found in more than one of the essential oils (i.e. limonene, geraniol and geranyl acetate), the highest concentration found was used. Since not all compounds found in the original essential oil were tested, a mixture of the tested compounds was made for every essential oil, with the same ratio as found in the original essential oil, to confirm the activity (see table 2).

Table 2. Summary of all compounds tested in the bioassays with *Bemisia tabaci* together their test concentration and oil of origin.

Compound	**Essential oil origin**	**Test concentration (mg/ml)**
Limonene	Citronella (3.34%) Lemongrass (1.9%)	0.25
Citronellal	Citronella (34.74%)	2.91
Citronellol	Citronella (12.03%)	1.02
Geraniol	Citronella (22.50%) Lemongrass (4.5%)	2.05
Geranyl acetate	Citronella (3.51%) Lemongrass (3.85%)	0.37
β-pinene	Cumin (12.19%)	0.87
ρ-cymene	Cumin (9.74%)	0.86
γ-terpinene	Cumin (11.59%)	0.85
Cuminaldehyde	Cumin (30.09%)	2.93
Cinnamaldehyde	Cinnamon (78.51%)	8.40
Cynnamyl acetate	Cinnamon (3.15 %)	0.32
2-methoxycinnamaldehyde	Cinnamon (9.65%)	0.90
Linalool	Lemongrass (0.69%)	7.14
Citral	Lemongrass (74.08%)	7.14
β-caryophyllene	Lemongrass (1.80%)	0.18
DEET	-	9.98
Permethrin	-	11.90

Testing

In order to gain a preliminary understanding of the potential for usefulness of the essential oil-derived compounds that are listed in table 2, several tests were carried out on toxicity, repellency and irritancy.

Toxicity test

Four hour toxicity

In order to test for the toxicity of a compound, individual polyethylene nets (AgroNet 0.9 NT; A to Z textile Mills Company, Arusha, Tanzania), with 40 holes/cm² and mesh size of about 0.9 mm, was treated with the 1% solution, the 0.1% solution and a 97% ethanol (control). A square piece of net of approximately 36 cm² was dipped into each compound solution for 5-10 seconds and then dried under a hood for at least 15 minutes. For each treatment 6 nets were prepared. Each net was used to separate two transparent plastic tubes (Dominique Dutscher SAS®; Ø 5 cm, length 10 cm; figure 2). One end of the system was closed and the other was used for ventilation, covered by a fine mesh that whiteflies could not cross. The tube with the closed end was covered with black paper and tinfoil such that it was completely dark inside (figure 2). Approximately 100-200 *B. tabaci* (of mixed sex and age) were placed in the dark tube, after which the entire system was placed in a climatic room with illumination and constant temperature and relative humidity (RH) (27 ± 1°C, 50 ± 10% RH) for 4 hours. As *B. tabaci* have an innate tendency to move towards light, they were attracted to the light part of the system, giving them an incentive to pass through the net. For each compound, all concentrations (i.e. 1%, 0.1% and control) and tube systems (6 per concentration) were tested at the same time. After 4 hours the experiment was stopped and the number of *B. tabaci* on each side of the net was counted to determine the cross rate. The results were analyzed with the Fisher's exact test . Since *B. tabaci* are attracted to light, the highest number of whiteflies was expected in the light part of

the tube for the control and if there was no effect of the compound. Also the total number of dead whiteflies was counted to determine the 4 hour mortality rate.

Twenty-four hour toxicity

All whiteflies that had crossed the net during the 4 hour toxicity test, and had thus been in direct contact with the compound, were collected in a Petri dish with a lid covered with agar gel (1%) and a tomato plant (*S. lycopersicum*) leaf. After 24 hours in the illuminated climatic room (27 ± 1°C, 50 ± 10% RH) the mortality rate was determined. The results were analyzed with the Fisher's exact test.

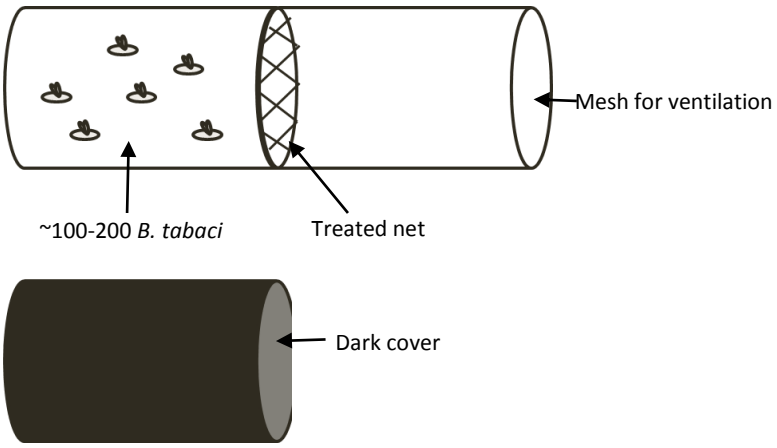


Figure 2, setup for the toxicity tes

Pass rate

The total number of *B. tabaci* on each side, dead or alive, were used to determine the cross rate after 4 hours. Although this test does not give information on the reason why *B. tabaci* do not cross the net (e.g. if they died or were repelled), but it gives an indication of which compounds can be potentially be useful to prevent them from crossing the net.

Repellency test

Olfactometer

For the first test for repellency, an olfactometer as described by Zhang et al. (2004) was used. It consisted of a glass tube (Legallais society®; length 30cm, Ø 3 cm) with a closed glass stopper on top, and a glass stopper pierced with a small tube for ventilation at the bottom. The tube was divided into three hypothetical zones; 2 cm from the top, the middle section, and the bottom 10 cm (see figure 3).

For each compound, 40 µl of compound solution or ethanol (control) was added to a square piece (4cm²) of non-weaving fabric filter paper. Immediately after the treated filter paper had dried (never longer than 5 minutes after applying the solution) it was placed in between the top stopper and the tube. To prevent *B. tabaci* from physically touching the solution, the paper was covered with a very small and dense mesh, which still allowed the volatile compounds to pass through it. The tubes were hung vertically in a dark hood. Due to the volatile properties of the compounds, a concentration gradient emerged within each tube, with the highest concentration at the top of the tube. After a short period (~1 minute) in the freezer (-20°C), to demobilize them, 10-20 whiteflies (of mixed sex and age) were placed in the lowest part of the tube by carefully letting them drop from their jar. A light shining from above attracted them to the top of the tube. After 1h the experiment was stopped and the number of individuals in each zone was counted. The number of dead whiteflies was also noted. Because of the tendency of whiteflies to go towards light, the highest number of whiteflies was expected to be found at the top in the control treatment, and when a compound has little or no repellent effect by the compound.

The concentrations of each compound (i.e. 1% and the 0.1% solutions) were tested in separate trials. In each trial, 4 tubes with the control solution and 4 tubes with the test solution were tested at the same time. In between trials the tube was rinsed with ethanol, dried and a new filter paper and cover would be used.

The distribution of the whiteflies in the different zones was compared between the control and the treated tubes that were tested in the same trial. The analysis was done with a Fisher's exact test.

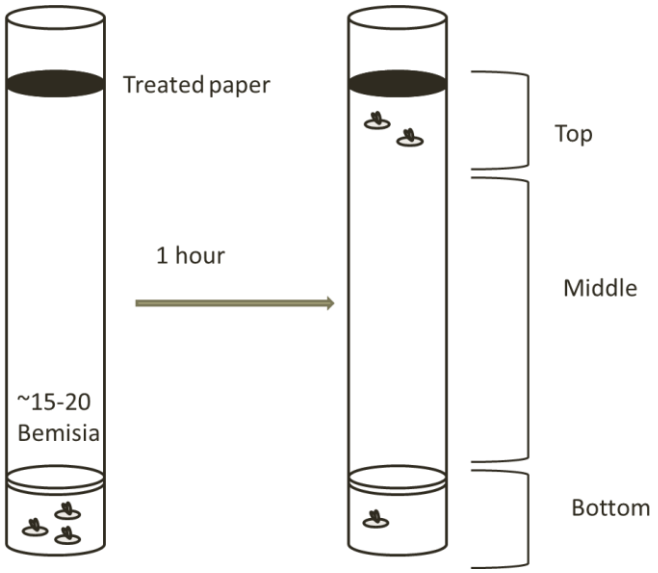


Figure 3. Method of the olfactometer repellency test

Choice tube test

A choice test was used to test if there is a repellent effect when the whiteflies are given the opportunity to move towards the light by crossing a non-treated net as well as a treated net. The set-up was similar to the toxicity test, with the addition of another tube between each end tube. Each of the three tubes was separated by a net. On one side the net was treated with a test compound, and on the other side it was treated with 97% ethanol (control). For the control test, both nets were treated with 97% ethanol. The tube in the middle was darkened by means of black paper and tinfoil. At the start of the experiment, the whiteflies were placed in the darkened tube after approximately 1 minute in the freezer. For each treatment (1%, 0,1% and control) 6 tubes were tested. The tubes were placed in a climatic room (27 ± 1°C, 50 ± 10% RH) and after 4 hours the number of whiteflies in each tube and the number of dead whiteflies were counted. It was expected that *B. tabaci* choose the site of the non-treated net if a compound is repellent, and for the control the expectation was that they distribute evenly across the two outer tubes. As the resulting distribution of whiteflies in the two outer tubes was not found to be 1:1 in the control, another series of tests was done to further investigate this unexpected result. Whiteflies were placed in either one or both outer tubes, with untreated nets separating the tubes. Then the test was repeated multiple three times with the tubes in different orientations. After this was done inside the climatic chamber, the same experiment was also carried in a hood in the laboratory, to check for environmental influences. In all trails, the distribution of *B. tabaci* did not approach a 1:1 ratio in the control, instead it seemed that the *B. tabaci* always went to one side. To which side they would go to differed between the replicates within and between trials. No pattern or explanation was found for this behavior. Therefore this experiment was terminated.

Irritancy test

The irritancy tests were only carried out on citronellal, geranyl acetate, cuminaldehyde, cinnamaldehyde, cinnamylacetate, 2-metoxycinnamaldehyde, citral, as well as on the positive controls DEET and permethrin. For all compounds, different to the other experiments, the paper was not treated with the amount that it is found in the essential oil, but 1% of the pure compound. Only for cinnamalehyde and cinnamylacetate, it turned out the *B. tabaci* would not move at all at that concentration. For these two compounds, the paper was treated with a 0,5% solution of the pure compound.

Choice test

In this test, for each compound a 15x12 cm section of black crepe paper was prepared to use as the "arena" by evenly dripping 2 ml compound solution (or 97% ethanol for the control) on to the surface before drying. The paper was always prepared on the same day that it was used, and between trials the paper was stored at -20°C. The arena was a 16 cm^2 square area with half of the surface consisting of the treated paper (the treated zone) and the other halve of the control paper (the control zone. On the sides of the arena a 2mm thick cardboard border and a Plexiglas cover prevented the whiteflies from escaping during the experiment. The *B. tabaci* were placed at the center of the paper, one per trial, and their activity (i.e. the time spent moving, average speed when moving, distance moved and the time spend in each zone) was observed for 10 minutes. The experiment was repeated 30 times with different individuals. After 5 recordings the paper on the floor was replaced and the orientation of the arena changed. The observations were made using a video camera fixed above the arena that recorded (25 frames per second) the activity and recordings were analyzed using the video observation system Ethovision (Noldus Information Technology, Wageningen, The Netherlands), a system used to automate animal behavioral observations (Noldus et al. 2001). The data was analyzed using a paired t-test or a Wilcoxon test (in the case of non-normally distributed data). For compounds with an irritant effect, an increased activity is expected in the treated zone. Also, for an irritant compound, it is expected that *B. tabaci* spend less time in the treated zone.

No choice test

In this test, a similar set-up was used as with the irritancy choice test. But now two square arenas were used, both 9 $cm^{2,}$ and the paper on the surface would either be treated or non-treated (control, 97% ethanol). The *B. tabaci* were placed in the arena, and their activity was recorded for 10 minutes. In total 20 recordings (of 20 individuals) were made, 10 for the treated zone and 10 for the control zone, per test compound. After 5 recordings, the paper on the floor was replaced and the orientation of the arena changed. The distance moved, the average velocity when moving and the mobility (time spent moving) in each zone were compared between the two zones. Results were analyzed using a unpaired t-test or a Wilcoxon test (in the case of non-normally distributed data). Again, an increased activity, compared to the control, is expected when the compound is irritant.

Data analysis

All data was analyzed with R version 2.15.2 (R Core team 2012), in which all graphs were also made. For all tests, a treatment was considered significantly different from the control when $p < 0.05$.

Results

Toxicity

All tested compounds and mixtures had a 4 hour toxicity at the high concentration, except for β-pinene. At the lowest concentration tested, an effect for the 4 hour toxicity was only found for the cumin, cinnamon and lemongrass mixtures and for citronellol and cinnamlyl acetate (figure 4). For the 24 hour toxicity test at the highest concentration, significant results were found for all four mixtures and compounds: cuminaldehyde, P-cymene, citral, geraniol, citronellal, citronellol, cinnamaldehyde and cinnamyl acetate. At the lower concentration, significant results were found for all mixtures except citronella grass and for cuminaldehyde, geraniol, citronellal and cinnamyl acetate (figure 4). At the higher concentration an effect on the cross rate was found for all mixtures and compounds except β-pinene, p-cymene and 2-metoxycinnamaldehyde. At the lower concentration, an effect was found for all four mixtures and compounds: citral, citronellol and cinnamyl acetate (figure 4). Positive control DEET showed significant results for the 4 and 24hour toxicity tests and for the cross rate at both 1% and 0.1% concentration. Permethrin however only showed significant results for the 1% concentration of the 4hour toxicity test and the cross rate (figure 4).

In order to estimate toxicity, the mortality after 4 hours of exposure, after 24 hours after the 4-hour exposure, as well as the cross rate was determined for the two concentrations of each compound. The average mortality (percentage) and the *p*-value (Fisher's exact test) for each compound can be found in Appendix I for both tested concentrations.

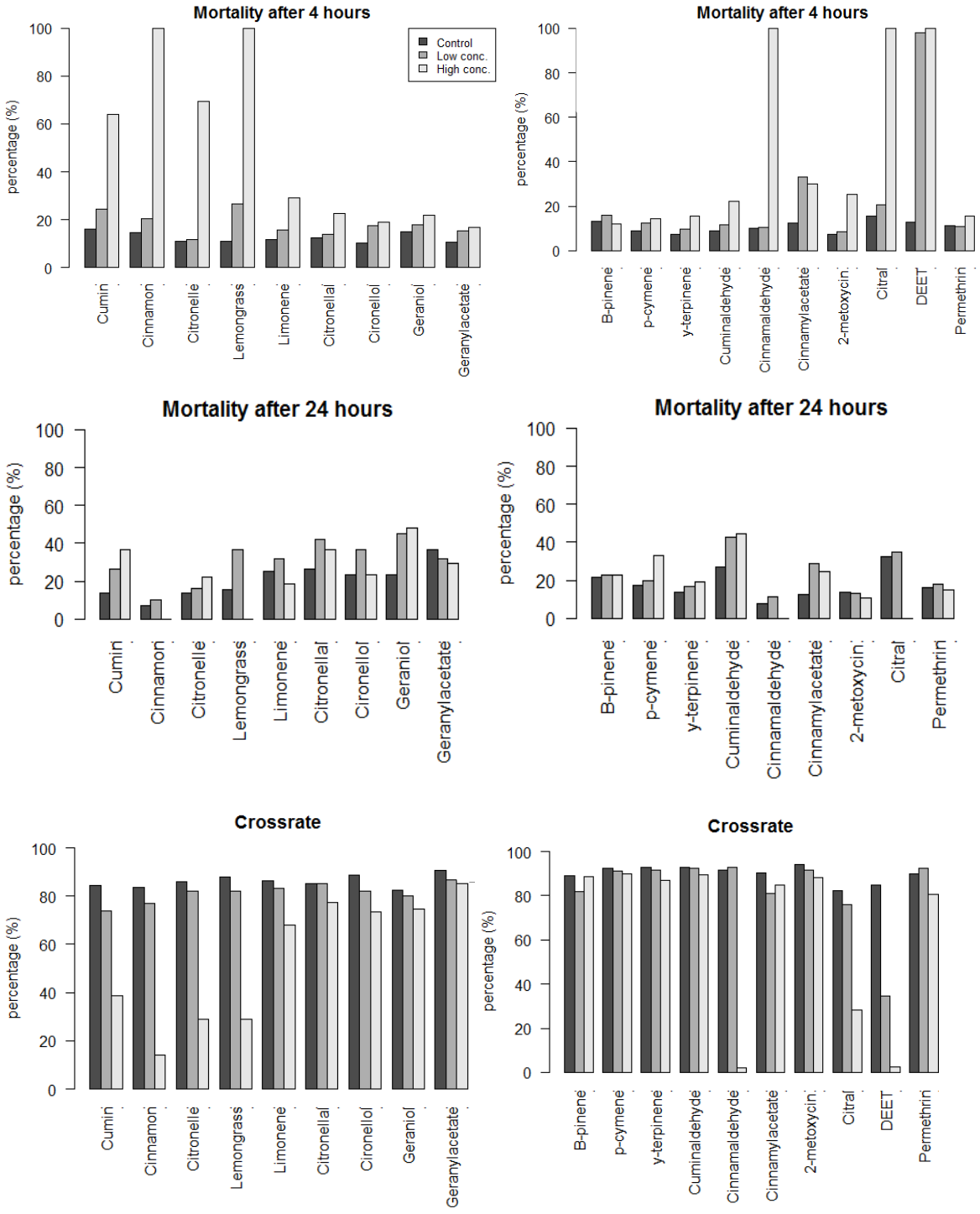


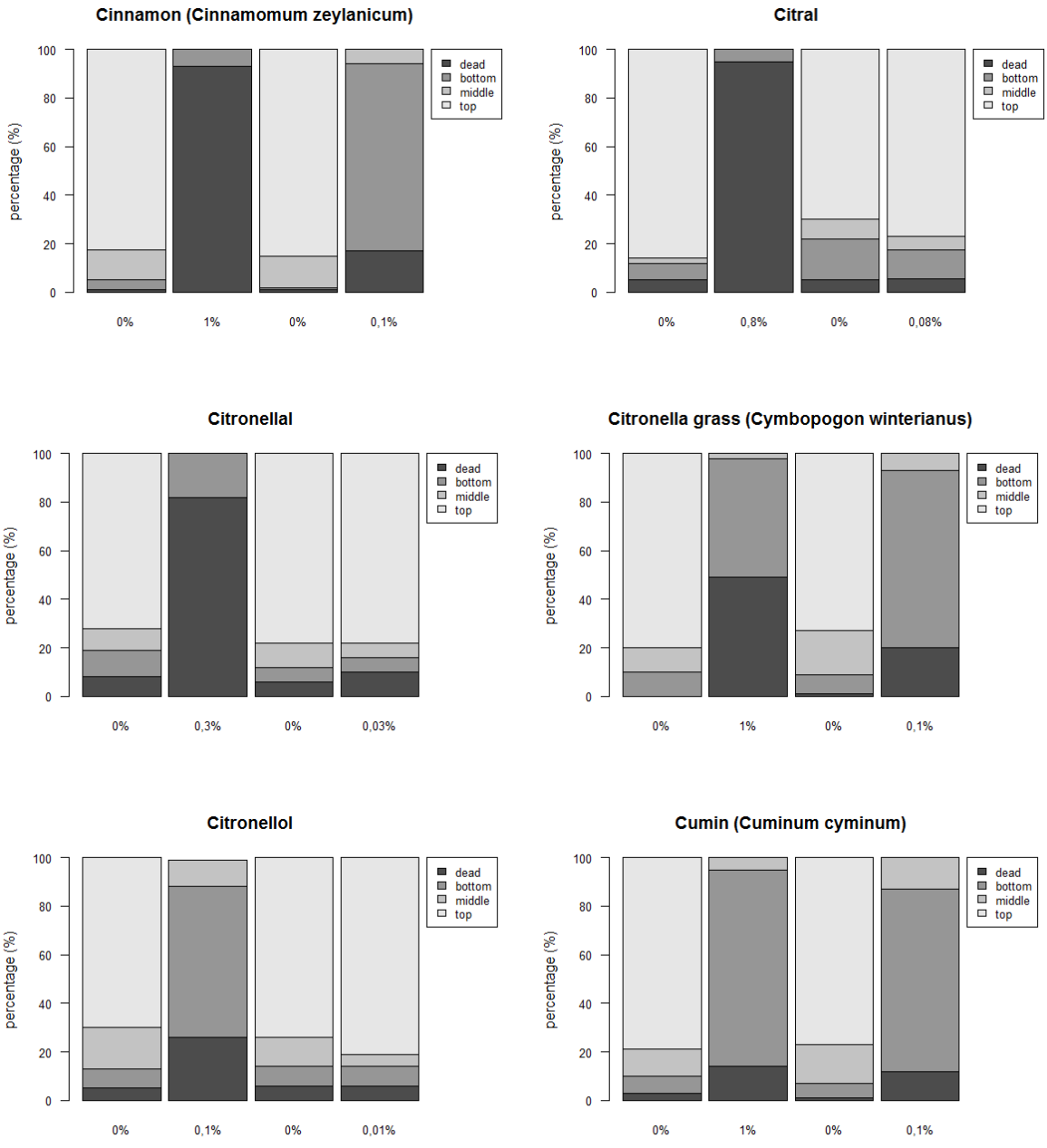
Figure 4. Toxicity of tested compounds. Results are shown for the 4 and 24 hour toxicity test and for the cross rate. The percentage of dead *B. tabaci* (for toxicity) or the percentage that crossed the net (for the cross rate) are shown for the high and low concentration and the control for each compound.

Repellency

For the repellency test, the distribution of *B. tabaci* in the control was compared with the distribution in the treated olfactometer. These values (percentage of *B. tabaci* in each zones) and their *p*-values are shown in Appendix II. Graphs of the significant results are shown in figure 5. A treatment compound was considered significantly different from the control when $p < 0.05$.

All tested mixtures had a significant effect for repellency in both the high (1%) as the low (0.1%) concentration. The individual compounds that had an effect for the highest tested concentration are: cuminaldehyde, citral, geraniol, limonene, linalool, citronellal, citronellol, geranyl acetate and cinnamaldehyde. Compounds that also showed an effect at the 0.1% concentration are: geraniol, limonene, linalool and cinnamaldehyde. Cinnamaldehyde also showed significant results for the 0.01% concentration.

The positive control DEET gave significant positive results for both the 1% and 0.1% concentration. Permethrin however did not show any repellent effect.



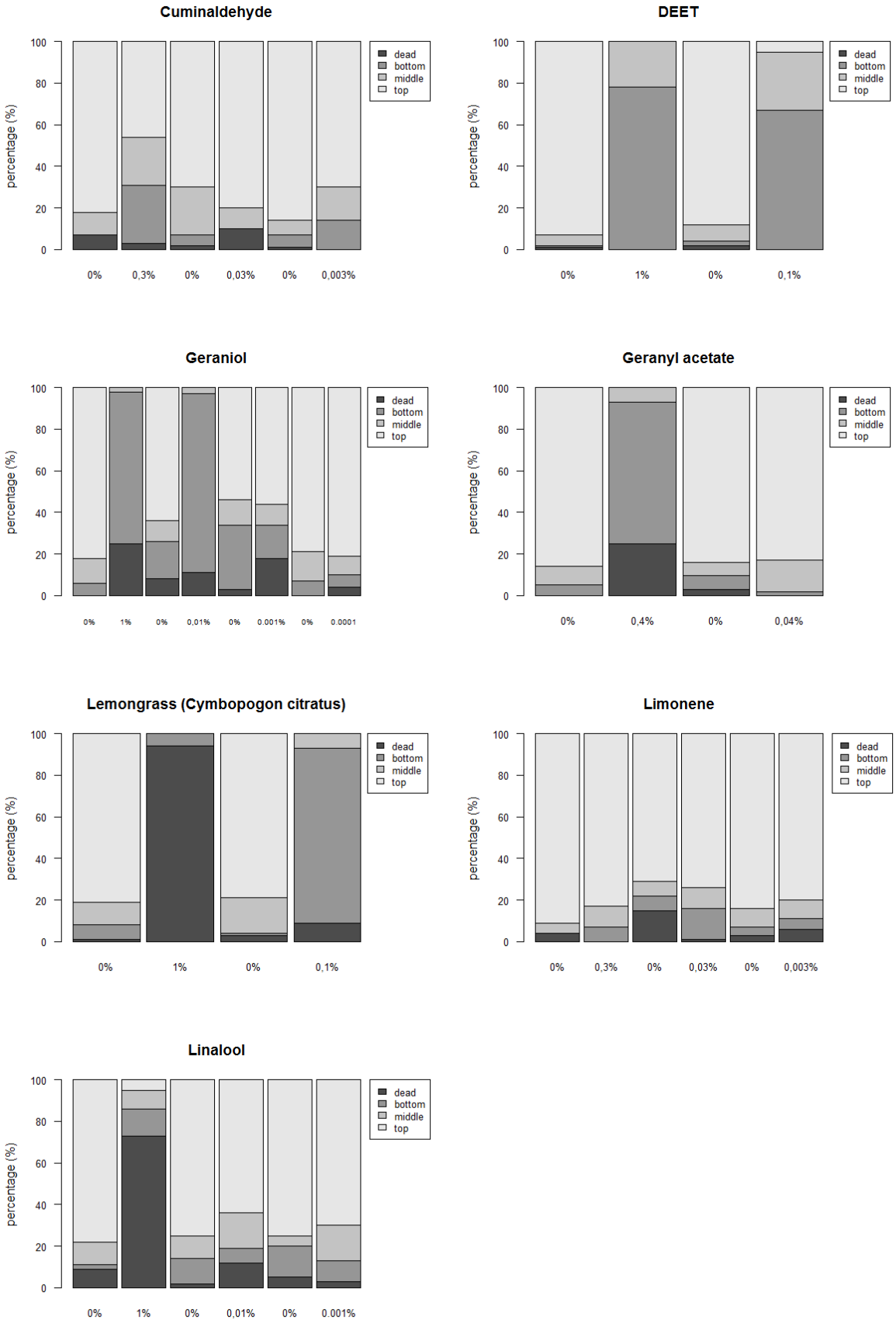


Figure 5.. Distribution of *B. tabaci* in the different zones of the olfactometer for the two tested concentrations of 13 test compound in the repellency test. The control for each concentration is denoted by '0%'. Only the compounds for which a significant results (Fisher's exact test, $p<0.05$) was found for the highest concentration tested are shown.

Irritancy

The results for the choice test are summarized in table 3 and figure 6, and the results for the no choice test in table 4 and figure 7. For both tests, no significant differences were found for all tested variables (average velocity when moving, mobility, distance moved and time spent in a zone) compared with the positive controls DEET and permethrin, making these results unreliable. The only two compounds that showed any significant results are citral and cinnamyl acetate, both in the no choice bioassay.

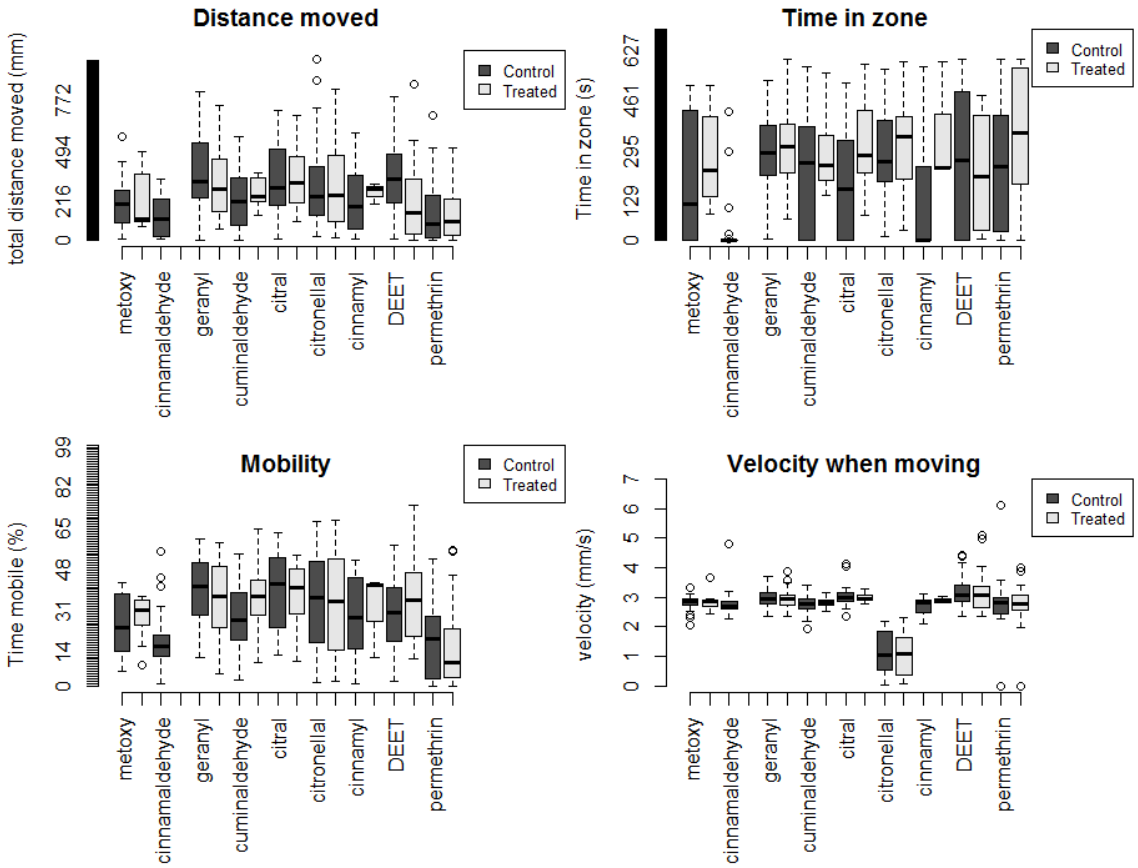


Figure 6. Results of the ethovision choice irritancy test. Boxplots of all tested compounds are shown for the variables: distance moved, time spent in each zone, mobility and average velocity when moving. For each compound both the control and treated results are shown.

Table 3. Results for the Ethovision choice irritancy test. For all tested compounds the average results of all replicates and *p*-values (paired t-test [1] or Wilcoxon's test[2]) are given for the distance moved, time spend moving (mobility), average velocity and the time spend in each zone.

Compound		Total distance moved (mm)		Mobility (%)		Average velocity (mm/s)		Time spend in zone (s)	
		Treated	Control	Treated	Control	Treated	Control	Treated	Control
2-metoxycinn.	Average	202.69	202.94	27.03	24.13	2.81	2.79	251.47	348.59
	p-value	0.643 [2]		0.527 [1]		0.7685 [2]		0.176 [1]	
Cinnamaldehyde	Average	146.10	127.81	17.64	18.87	2.80	2.76	358.68	241.40
	p-value	0.459 [2]		0.782 [2]		0.263 [2]		0.393 [1]	
Cinnamyl acetate	Average	200.37	226.17	24.80	32.61	2.71	2.82	176.70	305.37
	p-value	0.581 [1]		0.024 [2]		0.73 [2]		0.832 [1]	
Citral	Average	343.40	316.05	37.86	38.41	3.01	2.97	300.35	299.73
	p-value	0.564 [1]		0.833 [2]		0.29 [2]		0.991 [1]	
Citronellal	Average	298.55	307.96	34.21	35.44	1.07	1.14	312.74	287.34
	p-value	0.968 [2]		0.503 [2]		0.428 [2]		0.627 [1]	
Cuminaldehyde	Average	216.57	240.72	31.85	27.14	2.78	2.71	254.92	344.60
	p-value	0.395 [1]		0.173 [2]		0.062 [1]		0.117 [1]	
Geranyl acetate	Average	318.19	359.67	35.89	40.77	2.96	2.96	314.06	286.02
	p-value	0.385 [1]		0.005 [2]		0.971 [1]		0.543 [1]	
DEET	Average	256.63	337.40	35.24	30.05	3.19	3.19	247.04	353.05
	p-value	0.269 [2]		0.043 [2]		0.515 [2]		0.157 [1]	
Permethrin	Average	153.84	154.92	15.80	19.55	2.63	2.48	351.15	248.93
	p-value	0.570 [2]		0.184 [2]		0.39 [2]		0.205 [2]	

[1]Tested with a paired t-test.
[2] Tested with Wilcoxon's test.

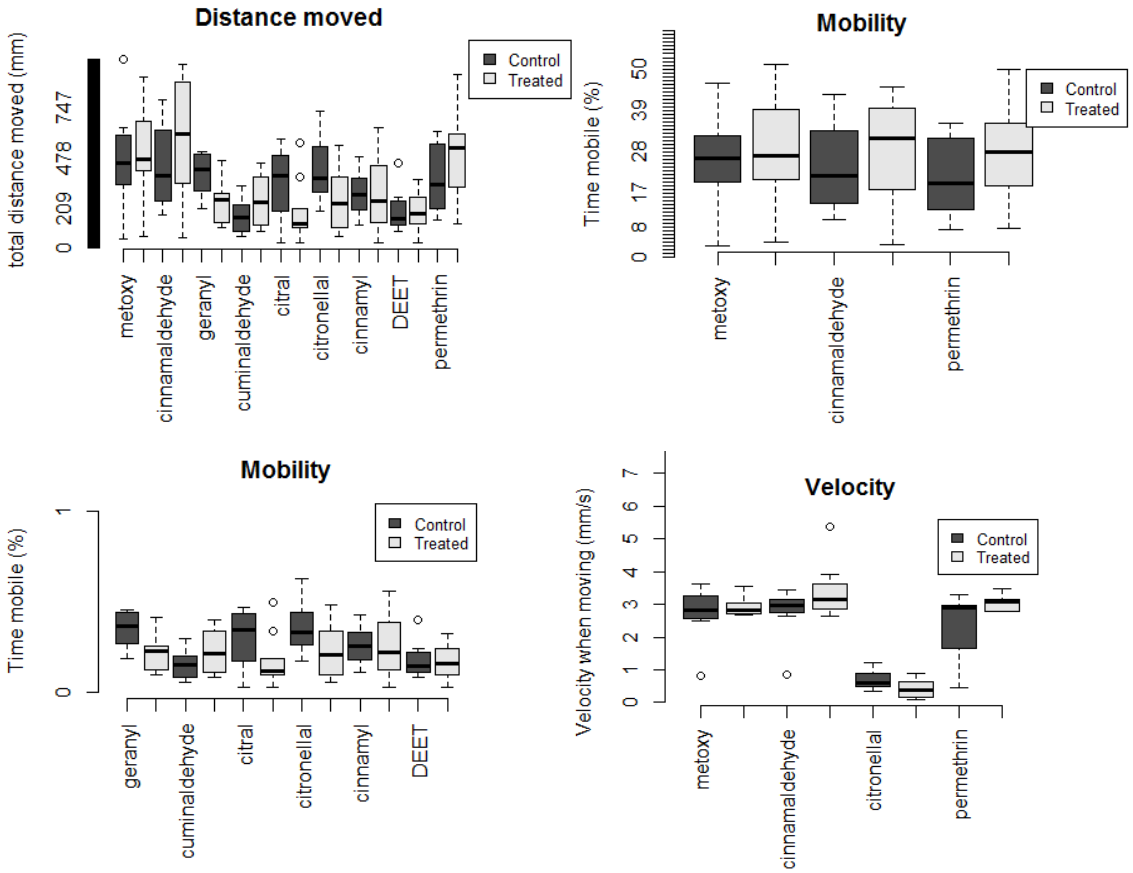


Figure 7. Results for the Ethovision no choice irritancy test. Boxplots of all tested compounds are shown for the variables: distance moved, time spent in each zone, mobility and average velocity when moving. For each compound both the control and treated results are shown.

Table 4. Results for the Ethovision no choice irritancy test. For all tested compounds the average results of all replicates and *p*-values (unpaired t-test [1] or Wilcoxon's test[2]) are given for the distance moved, time spend moving (mobility) and the average velocity.

Compound		**Total distance moved (mm)**		**Mobility (%)**		**Average velocity (mm/s)**	
		Treated	**Control**	**Treated**	**Control**	**Treated**	**Control**
2-metoxycinn.	Average *p*-value	472.04 0.870 [1]	450.43	27.50 0.84 [2]	24.18	2.84 0.44 [1]	2.97
Cinnamaldehyde	Average *p*-value	571.44 0.50 [1]	478.43	28.51 0.50 [2]	24.64	3.18 0.97 [2]	3.27
Cinnamyl acetate	Average *p*-value	273.80 0.83 [1]	290.20	0.24 0.72 [2]	0.26	189.03 0.04 [1]	188.39
Citral	Average *p*-value	152.66 0.02 [1]	345.06	0.14 0.04 [2]	0.30	188.46 0.08 [2]	190.56
Citronellal	Average *p*-value	253.38 0.11 [1]	393.89	0.22 0.14 [2]	0.34	0.42 0.11 [1]	0.66
Cuminaldehyde	Average *p*-value	249.67 0.21 [1]	179.26	0.22 0.19 [2]	0.16	188.55 0.34 [2]	188.42
Geranyl acetate	Average *p*-value	246.34 0.11 [1]	364.05	0.23 0.08 [2]	0.32	190.51 0.44 [2]	188.65
DEET	Average *p*-value	186.10 0.86 [1]	195.79	0.16 0.90 [2]	0.17	192.38 0.32 [2]	188.28
Permethrin	Average *p*-value	435.33 0.91 [1]	446.80	24.83 0.97 [2]	24.36	2.90 0.25 [1]	3.02

[1] Tested with an unparied t-test.
[2] Tested with Wilcoxon's test.

Overview

In table 5, a summary of the results for all tested mixtures and compounds is shown for the 4 hour and 24 hour toxicity, repellency and cross rate. The results from the irritancy test are not shown here, because these results are unreliable since the positive controls failed to show any results.

Table 5. Summary of the results of all compounds and mixtures for all tests. For each compound the tested concentration is given in parentheses, this is the same concentration as found in the essential oil of its origin. The mixture is the combination of the tested compounds of that essential oil (in the ratio in which they are found in the original essential oil). The outcomes of the tests (for the toxic (4hour and 24hour) effect, spatial repellent effect and cross rate) are shown in the columns. A significant effect found for a compound is presented by a cross (x). When there was also a significant effect found for the 10-times dilution of that compound, two crosses (xx) are given. A minus (-) means that no effect was seen for all tested concentrations. All tests were analyzed with a Fisher's exact test and the effects were considered significant for $p<0.05$.

Compound	Repellency	4 hour toxicity	24 hour toxicity	Cross rate
Cumin (mix of compounds)	xx	xx	xx	xx
Cuminaldehyde (30.09%) (2.93 mg/l)	xx	x	xx	x
β-pinene (12.19%) (0.872 mg/l)	-	-	-	-
γ-terpinene (11.59%) (0.85 mg/l)	-	x	-	x
p-cymene (9.74%) (0.86 mg/l)	-	x	x	-
Lemongrass (mix of compounds)	xx	xx	xx	xx
Citral (74.08%) (7.14 mg/l)	x	x	x	xx
Geraniol (4.5%)* (0.40 mg/l)	xx	x	xx	x
Limonene (1.9%)** (0.17 mg/l)	xx	x	-	x
β-caryophyllene (1.8%) (0.18 mg/l)	-	?	?	?
Linalool (0.69%) (0.06 mg/l)	xx	?	?	?
Citronella grass (mix of compounds)	xx	x	x	xx
Citronellal (34.74%) (2.91 mg/l)	x	x	xx	x
Geraniol (22.50%) (2.05 mg/l)	xx	x	xx	x
Citronellol (12.03%) (1.02 mg/l)	x	xx	x	xx
Geranyl acetate (3.51%) (0.37 mg/l)	x	x	-	x
Limonene (3.34%) (0.25 mg/l)	xx	x	-	x
Cinnamon (mix of compounds)	xx	xx	xx	xx
Cinnamaldehyde (78.51%) (8.4 mg/l)	xxx	x	x	x
2-metoxycinnamaldehyde (9.65%) (0.9 mg/l)	-	x	-	-
Cinnamylacetate (3.15%) (0.32 mg/l)	-	xx	xx	xx

*22.50% (2.05 mg/l) tested.

**3% (2.52 mg/l) tested.

Discussion

The aim of this study is to find out if individual compounds of four essential oils (viz. cumin, cinnamon, citronella grass and lemongrass) have a repellent, toxic and/or irritant effect on *B. tabaci* to be applied in pest control.

Most promising compounds

As explained earlier, a compound to be used in combination with a IPN would preferable be repellent from a distance, but not very toxic to prevent or at least delay the rapid development of resistance. The compound in this screening that fits these requirements best is cinnamaldehyde. This compound has a spatial repellent effect at a concentration of 0.084 mg/L, this was the lowest concentration tested for this compound, so the real effective concentration might be even lower. The toxic effect of cinnamaldehyde is low; the 4 and 24 hour toxicity test showed a toxic effect at a concentration of 8.4 mg/L.

Cinnamaldehyde is known to be toxic to *Tribolium castaneum*, *Trichoplusia ni* (Isman and Machial 2006) and *Coptotermes formosanus* (Chang and Cheng 2002). Also, some studies show repellent effects of cinnamaldehyde, e.g., the cat flea *Ctenocephalides felis* (Su et al. 2013), but not much research has been done on the repellent effect of cinnamaldehyde on insects. Cinnamaldehyde is widely used as a flavorant, in perfumes and in agriculture and is assumed to be safe to humans. However, apart from the LD50 in ginea pigs (1160 mg/kg), not much information is available on the potential ecological effects of this compound.

Another compound that is very repellent is linalool. In fact, linalool had an even lower effective concentration tested than Cinnamaldehyde for which it was spatially repellent; 0.006 mg/L. Because no toxicity assay was done on linalool, nothing can be said about the toxic effects on *B. tabaci*. In literature, linalool is considered to have insecticidal and fungicidal effects (Boulogne et al. 2012). Insects that are known to be affected by linalool are some coleopteran species (*Rhyzopertha dominica*, *Oryzaephilus surinamensis*, *Tribolium castaneum*, and *Sitophilus oryzae*) (Shaaya et al. 1991), larvae of the mosquito *Culex pipiens molestus* (Traboulsi et al. 2002) and the Mediterranean fruit fly (*Ceratitis capitata*), however these results were found for linalool in combination with lemon peel extracts (Salvatore et al. 2004).

Effect of compounds in the mixture

As expected, all mixtures, except citronella grass, of the abundant constituents of the four essential oils have significant results for all tested properties at both high and low concentrations. However, changes where found between compounds when tested individually. This confirms that it is likely that the total effect of the mixture is the result of a combination of the different effects of the compounds. At the highest concentration, most compounds are both repellent and toxic, but at the lower concentrations they are often only toxic or repellent. Also, for the mixtures of cumin and lemongrass, all compounds individually have a 4 hour toxic effect that is less than when mixed, suggesting an additive effect of these compounds.

For citronella grass, a different pattern is seen. Here the compounds separately have a higher toxic effect than when combined in the mixture. This was the case for both the 4 hour and the 24 hour toxicity. An explanation for this could be an antagonistic interaction between the compounds when mixed together, reducing their toxicity or even makes them loose the toxicity at all. If this is the case, citronellal, geraniol and citronellol would have to be targeted by this mechanism. If you look at the molecular structures of these molecules, they are very similar. They are also very similar to the compounds of lemongrass, but less saturated. It is possible that the fact that the mixtures toxicity is less has something to do with that. However, to be able to draw any conclusions on this, the role of the structural properties of these molecules on *B. tabaci* should be investigated more.

As positive controls, DEET and permethrin were used. DEET was toxic and repellent in all cases, except for the irritancy test. Permethrin, however, only showed significant results for the highest tested concentration (1%) in the 4 hour toxicity and cross rate. The permethrin used in each assay came from the same stock, therefore it is not possible to rule out that these effects where not caused by a lack of quality of this stock, making all results of permethrin unreliable. Another explanation could be that permethrin is not well suited to be a positive control for *B. tabaci*, because it has no or only little toxic and repellent effects on these whiteflies. In literature, there is no information available on the effect of permethrin on *B. tabaci* to confirm this.

Irritancy effect

No conclusions can be drawn on the irritancy effect of the compounds. This is due to the fact that this bioassay did not show any effect in most cases, including the positive controls. Therefore, no conclusions on the irritant effect of the compounds could be drawn. The lack of effect can be due to different factors, including the way the experiment was set up. The results show a high variance between individuals, making it hard to see differences between control and treated groups, this might be caused by the length of the observation period or the relatively small number of individuals tested. Also, it could be that the paper used as ground is not suited for this kind of experiments, this could be because of the relative rough surface, the lack of absorbing capacity or other properties. Also, sometimes it happened that the *B. tabaci* were seen walking on the Plexiglas covering the arena instead of on the ground, thereby avoiding contact with the ground and thus the compound. To test the effect of direct contact with the compound, it should not be possible for the whitefly to avoid that contact. To be able to get information about the irritant effect of compounds, these problems should be solved. This could be by making more and longer video's, or changing the ground and preventing the *B. tabaci* to lose contact with the substrate (e.g. by having the Plexiglas cover closer to the surface). What also may have caused the lack of results is that the *B. tabaci* were stressed during the experiment. If stress affects the movement (e.g. speed and amount of time spend moving) more than the irritant effect of the compound, it can mask the irritant effect of the compound. In the other experiments, stress does not seem to be a significant factor in the results, based on the fact that both the positive as negative control did not show any unexpected results. This can be because in those experiments, the changes of the behaviour caused by the stress are not essential for the test. Also, all other experiments lasted longer than the irritancy test.

Future directions

In this project, the focus was on finding a substance that is repellent to *B. tabaci* to protect crops from their damage, but that avoids the rapid occurrence of resistance to the compound. To accomplish that, it seems evident that for this compound there should to be no, or very little, selective pressure. However, recent studies have shown that selection is not always the only way organisms become resistant to repellent

effects of compounds. A study on *Rhodnius prolixus* showed behavioral changes in fifth instar nymphs after the exposure to DEET, resulting in a decrease in repellency (Sfara et al. 2011). Another study, on *Aedes aegypti*, showed behavioral insensitivity to DEET after pre-exposure and this was explained by the observed decrease in response by the the olfactory receptor neurons to DEET (Stanczyk et al. 2013). If similar processes to a repellent also occur in *B. tabaci*, the effect of using a compound that is only repellent (and not toxic) might even be less than when using a compound which is both. Because if they manage to cross the net and stay there without being killed, they are protected against predators. To be certain that the chosen repellent is suitable in practice, it might be worth to look in more detail at the behavioral resistance in *B. tabaci* in relation to the repellent.

The laboratory experiments only provide a direction and a screening method on which products could potentially work in the field. To really know how effective these compounds will be, tests should be performed in the field. In a laboratory it is impossible to simulate all factors that are operational in the field, such as wind, sun, other odors, etc. Especially since all tested compounds are very volatile compounds, there is a risk that they might only work for a short period of time before they are no longer detectable by the insects.

Another aspect to look at is the safety of the chosen compound, both to human as to the environment in general. Although essential oils and their compounds are natural products and therefore often considered safe, that is not necessarily the case. Compounds can have unexpected effects, especially when used in much higher concentrations than would occur naturally. The fact that most of the compounds showed toxic effects on *B. tabaci* indicates that they might well be toxic to other organisms as well. So before the widespread use in the field, this should be examined carefully first.

There is also the possibility to look at other approaches than to use chemicals on the net to repel whiteflies. One of them, which seems promising, is intercropping. This way you can use the plant in which the repellent naturally occurs to provide the effect, thereby reducing the amount of chemicals or biopesticides needed for crop protection. Plants that would be useful intercrops should have the same growth and condition requirements as the plants it is intercropped with (which is in this case the tomato). Also the compound should be available in the parts that are above the soil and preferably all year around. In this case plants that contain cinnamaldehyde and/or linalool in relative high amounts could be useful to investigate, if they match the requirements for intercropping. Since cinnamaldehyde is only found in high amounts in the bark of trees, this is not really an option. Linalool however can be found in the shoots of *Origanum sipyleum* at a concentration of 0-35 ppm (Baser et al. 1992) and this might be a plant worth investigating.

Finally, it is important to understand the mechanism behind the repellent and toxic effects, especially of cinnamaldehyde and linalool, on *B. tabaci* This can help predict the effects in the long term, but also help to choose alternatives in the future. To help do this, the irritancy test should be improved, as mentioned earlier, or a completely different setup could be designed and tested. Also, the strange behavior in the tube-choice-test could be looked at in more detail, to provide more information on the factors that motivate *B. tabaci* to go into a certain direction. This could be done by eliminating all possible factors (such as light, wind, odors, etc.) as much as possible and then varying them one at a time. Also, more data about the physiology and how that is affected by the different compounds should be collected. This can best be done by comparing the effects of compounds that have one dominant effect (i.e. toxic or repellent) on *B. tabaci*.

Acknowledgements

I would like to thank my supervisors, for their help with my daily work, advice and support.

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Appendix I. Toxicity after 4h, 24h and cross rate.

Compound	Concentration (%) (mg/L)	Mortality after 4h (%)	*p*-value*	Mortality after 24h (%)	*p*-value*	Cross rate (%)	*p*-value*
Limonene	0	11,74		25,09		86,54	
	0.03 (0.25)	15,87	0,057	31,56	0,226	83,28	0,104
	0.3 (2.52)	29,10	<0,001	18,69	0,193	67,86	<0,001
Citronellal	0	12,52		26,22		85,34	
	0.034 (0.29)	13,73	0,566	41,91	<0,001	85,24	1
	0.34 (2.91)	22,59	<0,001	32,65	0,096	77,30	<0,001
Citronellol	0	10,11		23,36		88,76	
	0.012 (0.10)	17,43	<0,001	36,51	0,001	82,22	<0,001
	0.12 (1.03)	19,07	<0,001	36,46	0,001	73,42	<0,001
Geraniol	0	14,86		23,69		82,50	
	0.023 (0.20)	17,70	0,239	45,27	<0,001	80,17	0,288
	0.23 (2.05)	21,79	<0,001	48,22	<0,001	74,49	<0,001
Geranyl acetate	0	10,4		36,59		90,67	
	0.04 (0.37)	15,38	0,079	31,82	0,666	86,61	0,100
	0.40 (3.66)	16,77	0,037	29,33	0,489	85,03	0,027
β-pinene	0	13,36		21,95		89,17	
	0.01 (0.09)	15,96	0,396	22,99	0,900	81,80	<0,001
	0.10 (0.87)	12,03	0,621	23,11	0,904	88,82	0,915
ρ-cymene	0	8,82		17,61		92,61	
	0.01 (0.09)	12,31	0,139	19,67	0,637	91,00	0,405
	0.10 (0.86)	14,58	0,013	32,82	0,003	89,94	0,173
γ-terpinene	0	7,49		13,66		93,03	
	0.01 (0.09)	9,92	0,186	16,73	0,459	91,04	0,241
	0.10 (0.85)	15,38	<0,001	19,05	0,235	86,92	<0,001

Compound	Concentration (%) (mg/L)	Mortality after 4h (%)	*p*-value	Mortality after 24h (%)	*p*-value	Cross rate (%)	*p*-value
Cuminaldehyde	0	8,83		27,30		93,04	
	0.03 (0.29)	11,47	0,205	42,46	0,008	92,47	0,733
	0.3 (2.93)	22,22	<0,001	44,42	<0,001	89,53	0,027
Cinnamaldehyde	0	10,18		7,88		91,76	
	0.08 (0.84)	10,63	0,855	11,59	0,172	92,66	0,599
	0.8 (8.40)	100,00	<0,001			2,10	<0,001
Cinnamyl acetate	0	12,32		12,30		90,48	
	0.003 (0.03)	33,28	<0,001	28,72	<0,001	81,19	<0,001
	0.03 (0.32)	29,96	<0,001	24,40	0,001	84,93	0,005
2-metoxycinnamaldehyde	0	7,19		13,71		94,10	
	0.009 (0.09)	8,38	0,360	13,03	0,785	91,84	0,785
	0.09 (0.90)	25,28	<0,001	10,70	0,231	88,06	0,231
Citral	0	15.71		32.24		82.47	
	0.08 (0.71)	20.74	0.052	35.15	0.578	76.01	0.004
	0.8 (7.14)	100	<0,001			28.20	<0,001
Cumin[1]	0	16,08		13,51		84,47	
	0.1	24,54	0,015	26,49	0,007	74,02	<0,001
	1	63,94	<0,001	36,59	<0,001	38,68	<0,001
Cinnamon[2]	0	14,70		7,16		83,78	
	0.1	20,26	0,030	10,22	0,197	76,97	0,002
	1	100,00	<0,001			14,19	<0,001
Citronella[3]	0	11,04		14,03		86,07	
	0.1	11,68	0,714	16,09	0,447	82,24	0,028
	1	69,55	<0,001	22,50	0,023	28,95	<0,001
Lemongrass[4]	0	10,97		15,71		88,12	
	0.1	26,62	<0,001	36,65	<0,001	70,69	<0,001
	1	100,00	<0,001			4,51	<0,001

Product	Concentration	Mortality after 4h (%)	*p*-value	Mortality after 24h (%)	*p*-value	Cross rate (%)	*p*-value
DEET	0	12.78				84.74	
	0.1 (0.998)	97.88	<0,001			34.44	<0,001
	1 (9.98)	100	<0,001			2.67	<0,001
Permethrin	0	11.40		16.02		89.89	
	0.1 (1.19)	10.90	0.127	17.82	0.637	92.44	0.127
	1 (11.9)	15.60	<0,001	15.33	0.845	80.69	<0,001

[*] *p*-values were determined with a Fisher's exact-test in R.

[1] Cumin mixture: 30.09% cuminaldehyde, 12.19% β-pinene, 11.59% γ-terpinene, 9.74 p-cymene.
[2] Cinnamon mixture: 78.51% cinnamaldehyde, 9.65% 2-metoxycinnamaldehyde, 3.15% cinnamylacetate.
[3] Citronella mixture: 34.74% citronellal, 22.50% geraniol, 12.03% citronellol, 3.51% geranyl acetate, 3.34% limonene.
[4] Lemongrass mixture: 74.08% citral, 4.5% geraniol, 1.9% limonene.

Appendix II. Repellency test. For each compound, the percentage *B. tabaci* in each zone is given for all concentrations. The distribution within the control is also given.

			Control				Treated			
Product	Conc (%) (mg/L)	*p*-value*	Top (%)	Middle (%)	Bottom (%)	Dead (%)	Top (%)	Middle (%)	Bottom (%)	Dead (%)
Limonene	0.003 (0.03)	0,793	84.4	9.1	3.9	2.6	79.8	8.9	5.1	6.3
	0.03 (0.25)	0,011	70.9	7.3	7.3	14.6	74.1	9.4	15.3	1.2
	0.3 (2.52)	0,045	91,1	5.4	10.0	3.6	82.9	10.0	7.1	0.0
Citronellal	0.034 (0.29)	0,852	78.0	10.0	6.0	6.0	78.0	6.0	6.0	10.0
	0.34 (2.91)	<0,001	71.7	9.4	11.3	7.6	0.0	0.0	17.5	82.5
Citronellol	0.012 (0.10)	0,427	73.6	12.5	8.3	5.6	81.5	4.6	7.7	6.2
	0.12 (1.03)	<0,001	69.8	17.5	7.9	4.8	0.0	11.3	62.3	26.4
Geraniol	0.002 (0.02)	0,269	79.0	13.6	7.4	0.0	80.8	9.1	6.1	4.0
	0.023 (0.20)	0,005	54.3	12.4	30.9	2.5	55.9	10.3	16.2	17.7
	0.23 (2.05)	<0,001	63.9	9.7	18.1	8.3	0.0	3.0	86.36	10.6
	1 (8.89)	<0,001	82.0	12.0	6.0	0.0	0.0	1.7	72.9	25.4
Geranyl ac.	0.04 (0.37)	0,182	83.9	6.5	6.5	3.2	83.0	15.1	1.9	0.0
	0.40 (3.66)	<0,001	86.0	9.3	4.7	0.0	0.0	6.7	68.3	25.0
β-pinene	0.01 (0.09)	0,986	80.0	8.0	6.7	5.3	85.3	8.2	1.6	4.9
	0.10 (0.87)	0,679	76.2	13.1	8.3	2.4	74.7	13.3	9.6	2.4
ρ-cymene	0.01 (0.09)	0,126	75.8	9.9	12.1	2.2	82.9	5.3	10.5	1.3
	0.10 (0.86)	0,679	69.2	18.5	12.3	0.0	56.0	18.7	25.3	0.0
γ-terpinene	0.01 (0.09)	0,429	78.5	10.1	8.9	2.5	90.2	1.6	8.2	0.0
	0.10 (0.85)	0,105	90.5	5.4	4.1	0.0	82.6	8.7	5.8	2.9
Cuminaldehyde	0.003 (0.03)	0,095	85.7	7.1	5.7	1.4	70.6	15.7	13.7	0.0
	0.03 (0.29)	0,009	70.0	23.3	5.0	1.7	80.0	10.0	0.0	10.0
	0.3 (2.93)	<0,001	82.0	11.5	0.0	6.6	46.1	22.5	28.1	3.4
Cinnamaldehyde	0.008 (0.08)	<0,001	73.2	15.9	9.8	1.2	0.0	7.3	59.8	32.9
	0.08 (0.84)	<0,001	73.5	18.1	7.2	1.2	0.0	4.4	65.2	30.4
	0.8 (8.40)	<0,001	76.1	11.3	12.7	0.0	0.0	1.8	66.1	32.1
Cinnamyl acet.	0.003 (0.03)	0,145	73.4	15.6	4.7	6.3	88.9	9.3	0.0	1.9
	0.03 (0.32)	0,986	67.27	14.6	10.9	7.3	68.3	15.0	11.7	5.0

			Control				Treated			
Product	Conc (%) (mg/L)	*p*-value	Top (%)	Middle (%)	Bottom (%)	Dead (%)	Top (%)	Middle (%)	Bottom (%)	Dead (%)
2-metoxycin.	0.009 (0.09)	0.823	87.3	6.4	2.7	3.6	89.5	4.8	3.8	1.9
	0.09 (0.90)	0.780	89.8	5.1	4.1	1.0	86.0	5.6	5.6	2.8
Citral	0.08 (0.71)	0.772	70.3	7.8	17.2	4.7	77.0	5.4	12.2	5.4
	0.8 (7.14)	<0,001	86.0	1.8	7.0	5.3	0.0	0.0	5.4	94.6
Cumin[1]	0.1	<0,001	77.0	16.2	5.4	1.4	0.0	13.2	75.0	11.8
	1	<0,001	79.5	11.0	6.9	2.7	0.0	5.2	80.5	14.3
Cinnamon[2]	0.1	<0,001	84.2	13.2	1.3	1.3	0.0	6.5	76.6	16.9
	1	<0,001	82.5	12.5	3.8	1.3	0.0	0.0	7.2	92.8
Citronella[3]	0.1	<0,001	73.4	17.7	7.6	1.3	0.0	7.14	73.21	19.6
	1	<0,001	80.3	9.9	9.9	0.0	0.0	2.9	48.6	48.6
Lemongrass[4]	0.1	<0,001	78.7	17.3	1.3	2.7	0.0	7.14	84.3	8.6
	1	<0,001	81.3	10.7	6.7	1.3	0.0	0.0	6.5	93.5
DEET	0.1 (0.998)	<0,001	92.9	4.7	1.2	1.2	0.0	21.8	78.2	0.0
	1 (9.98)	<0,001	87.9	8.1	2.0	2.0	5.1	28.3	66.7	0.0
Permethrin	1 (11.9)	0.055	86.7	10.6	0.9	1.8	80.0	14.4	5.6	0.0
β-caryophyllene	0.02 (0.18)	0.130	60.9	12.5	26.6	0.0	45.3	25.0	29.7	0.0
Linalol	0.001 (0.01)	0.101	74.7	5.3	14.7	5.3	69.6	16.5	10.4	3.5
	0.01 (0.09)	0.038	75.0	10.9	12.5	1.6	63.4	17.1	7.3	12.2
	1 (8.58)	<0,001	77.8	11.1	1.6	9.5	4.7	9.3	12.8	73.3

[*] *p*-values determined with Fisher's exact-test.

[1] Cumin mixture: 30.09% cuminaldehyde, 12.19% β-pinene, 11.59% γ-terpinene, 9.74 p-cymene.
[2] Cinnamon mixture: 78.51% cinnamaldehyde, 9.65% 2-metoxycinnamaldehyde, 3.15% cinnamylacetate.
[3] Citronella mixture: 34.74% citronellal, 22.50% geraniol, 12.03% citronellol, 3.51% geranyl acetate, 3.34% limonene.
[4] Lemongrass mixture: 74.08% citral, 4.5% geraniol, 1.9% limonene.